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Evaluation of the antifungal effect and adhesive strength of a denture adhesive supplemented with nystatin

Avaliação do efeito antifúngico e da força adesiva de um adesivo protético suplementado com nistatina

Evaluación del efecto antifúngico y la fuerza adhesiva de un adhesivo protésico complementado con nistatina

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ABSTRACT

Introduction: The scientific basis related to the effect of incorporating antifungals on the properties of denture adhesives is scarce. **Aim:** This study incorporated an antifungal agent into an adhesive and evaluated the influence on biofilm formation and adhesive strength. **Design:** Specimens in resin based on polymethylmethacrylate (PMMA) were divided into groups: PMMA (No Adhesive); PMMA+Ultra Corega Cream Adhesive and PMMA+Ultra Corega Cream Adhesive+Nystatin. Biofilm of *Candida albicans* was grown on the specimens and the cell viability was investigated by counting colony forming units (CFU/mL). Adhesive strength was tested after 5 minutes, 6 and 12 hours. For microbiological analysis, data were evaluated by Kruskal-Wallis and Dunn's post test, and for analysis of adhesive strength by two-way ANOVA and Bonferroni post test (α=.05). **Results:** There was a reduction in biofilm formation on the surface of the nystatin-modified adhesive (p<.001). After 5 minutes, the adhesive strength of the modified Ultra Corega Cream was greater than that of the commercial product (p=.048), with no significant difference in the other times (p>.05). **Implications:** The incorporation of nystatin on denture adhesive reduced biofilm accumulation, with a positive influence on the initial adhesive strength, it may be a viable alternative for delivering the drug to the patient.

DESCRIPTORS

Adhesives; *Candida albicans*; Dental Prosthesis; Nystatin.

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INTRODUCTION

Conventional complete dentures remain the primary method for restoring fully edentulous arches.[1] However, even the most experienced professionals are often unable to meet the expectations of patients who complain of functional problems related to lack of retention and stability of the dentures, and psychological problems that interfere in their quality of life.[2-3] Therefore, adhesives for prostheses are materials commonly used by these patients.[4]

These materials improve the interfacial surface tension between the base of the denture and the soft tissues, increasing the retention and consequently the patient comfort.[5-6] Faced with these factors, denture adhesives are gaining ground and supporting a positive opinion among users.[7]

Denture stomatitis is a chronic disease that affects many denture wearers. It is characterized by an inflammation of the oral mucosa that is difficult to treat, due to its multifactorial etiology.[8-9] *Candida albicans* represents the main etiological agent,[10-11] and treatment includes adequate oral hygiene, associated with antifungal prescription.[12] Nystatin is the most popular agent in the treatment of infections caused by *C. albicans*, with fungicidal and fungistatic action.[13]

Regarding its mechanism of action, this polyenic antifungal binds to the ergosterol of the fungus plasma membrane and increases membrane permeability through the creation of pores, promoting the extravasation of essential cell components and, consequently, cell death.[14]

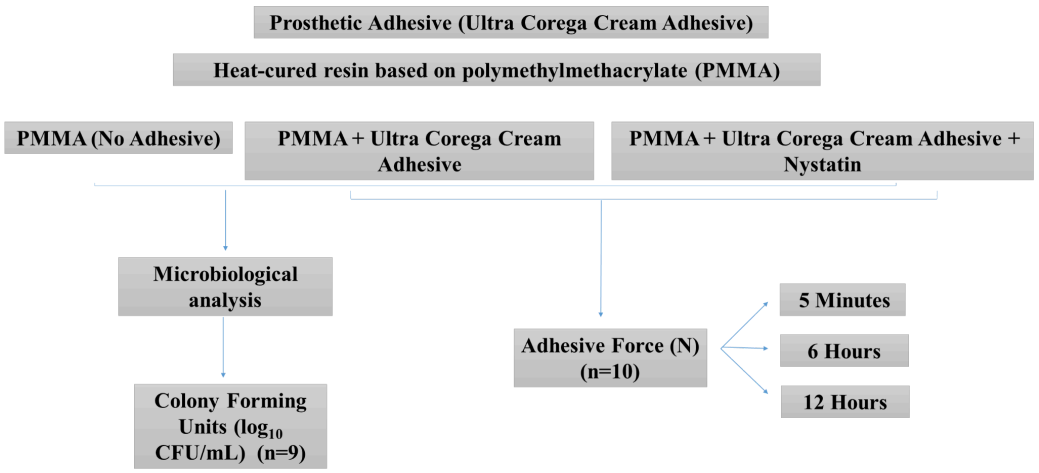
However, this approach has some limitations. In the oral cavity the salivary flow, as well as tongue movements and swallowing dissolve and eliminate the drug rapidly.[12] Thus, the use of the denture adhesive associated with antifungal compounds, for a limited period, may be beneficial in the treatment of denture stomatitis.[15]

Therefore, in this study, the feasibility of incorporating nystatin in a denture adhesive was evaluated. The null hypothesis was that the incorporation of the antifungal agent would have no influence on the formation of the biofilm and on the adhesive strength of the material used.

METHOD

The factor under study was the use or no of the denture adhesive, modified or not with nystatin. For microbiological analyses, the quantitative response variable was the amount of biofilm on the surface of the specimens, evaluated by colony forming units count in $\log_{10}$ (CFU/mL). For the mechanical analysis, the quantitative response variable was the adhesive strength (N), at 5 minutes, 6 hours and 12 hours. Figure 1 shows the study flowchart (Fig. 1).

Figure 1. Study flowchart



Source: Own authorship.

Preparation of specimens

A heat-cured polymethyl methacrylate acrylic resin was used (Classical Dental Articles). Initially, the wax patterns were included in type IV plaster (Gesso Rio), in metallic flasks (OGP; Odontological Products Ltda).

During the plastic phase, the resin was accommodated in the molds and the flasks were placed in hydraulic presses (Protecni hydraulic press, Protecni Equip. Med., Araraquara, SP, Brazil) with a load of 1000 Kgf for 60 minutes.[16] The samples were polymerized by conventional heating, according to the manufacturer's instructions in an electric thermocycler (Thermocycler T100, Ribeirão Preto, Brazil).[17] After disinclusion, the specimens were finished and stored in distilled water at 37ºC for 24 hours. In the end, 20 cylindrical specimens (25 mm in diameter × 35 mm in height) were obtained for the analysis of adhesive strength and 30 with a rectangular shape (6 mm wide x 10 mm long x 2 mm thick), for the analysis of antimicrobial activity.[18]

The specimens was standardized with an average surface roughness (Ra) of 3.0 µm with sandpaper, aiming to reproduce inner surface of the bases of the prostheses.[18-19] The roughness was evaluated using a roughness meter (Surftest SJ 201P; Mitutoyo Corporation) with read speed of 0.5mm/s and read length of 4.0 mm.

Microbiological analysis

Candida albicans (ATCC 10231) was used in the present study. Microbial colonization, including biofilm formation on substrates was evaluated. The substrates consisted in PMMA (No Adhesive); PMMA + Ultra Corega Cream Adhesive and PMMA + Ultra Corega Cream Adhesive + 100,000 IU/g nystatin (Table 1).

Table 1. Denture adhesive and antifungal used in the study

Material	Manufacturer	Composition
Ultra Corega Cream Adhesive	GSK	Poly sodium-calcium salts (methylvinylether/maleic acid) Carboxymethylcellulose Mineral oil Vaseline
Nystatin	Homeocenter Homeopathic Pharmacy with Manipulation, Ribeirao Preto	

Source: Own authorship - Data obtained from manufacturers.

The specimens were previously sterilized with hydrogen peroxide (Multilav Sterilization),[20] and then the adhesive was applied in a Class II biological safety cabinet (Pachane; Pa 400-ECO).

The amount of prosthetic adhesive with or without antifungal was standardized at 0.025 g. The adhesive was applied with a spatula and spread directly over the sample surfaces, forming a thin layer. After application, the samples were exposed to ultraviolet light for 20 minutes to disinfect the patch.[18-19] For the supplemented groups, the patch was exposed to ultraviolet light prior to the incorporation of nystatin, since the antifungal is sensitive to light.[21] Nystatin was weighed and incorporated into the adhesive by rubbing the glass plate and the material applied to the surface of the samples.

For the preparation of the inoculum, the microorganism was reactivated in Petri dish with the culture medium Sabouraud Dextrose Agar (HiMedia Laboratories; Pvt. Ltd.) and incubated at 37ºC for 48 hours. Next, standardized microbial inoculars (10^6 CFU/mL) were obtained in 0.85% saline solution using a PCB 687 spectrophotometer (BYK Gardner).

In a Class II biological safety cabin (Pachane; Pa 400-ECO), the specimens were individually

inserted into each well of a polystyrene plates of 24 wells (TPP; Trasadingen) with 1 mL of inoculated culture medium for biofilm formation. The plates were incubated at 37ºC for 1 h and 30 minutes under agitation at 750 rpm in a bacteriological incubator (Incubator Shaker, Mod. – CE-320, Cienlab, Campinas, SP, Brazil) for adherence of the microorganism to the specimens. After the adhesion period, the specimens were washed with 2 mL of 0.85% saline solution in order to remove non-adhered cells, buffer the medium and remove metabolites.

Then, 2 mL of sterile culture medium was added to each well to promote the growth of adhered microorganisms and biofilm maturation. The plates were incubated at 37ºC under shaking at 750 rpm for 24 h. After this period, the specimens were removed from the plates, washed with saline solution to remove planktonic cells, and transferred separately to the test tubes containing 3 mL of saline solution.

The test tube/specimen was taken to an ultrasound vat (200W, 40 kHz) (Altsonic, Clean 9CA, Ribeirão Preto, São Paulo, Brazil) for 20 minutes, and then serial dilution was performed. After seeding, the Petri dishes were incubated at 37°C for 24 hours and the number of viable cells was quantified in CFU/mL (n=10) and converted to $\log_{10}$.[18]

Adhesive force

Adhesive strength was measured using two cylinders specimens of acrylic resin.[18,22] One of the cylinders was moistened with tap water and 0.3 g of the adhesive (Ultra Corega Cream and Ultra Corega Cream + nystatin) were applied to each specimen. The samples were immersed in distilled water at 37°C for 5 minutes, 6 hours and 12 hours. Next, the other sample in the set was moistened with artificial saliva and then the two cylinders were aligned on the Universal Testing Machine (EMIC 1000). A compression force of 12 N was applied for 30 seconds, and then the tensile test was performed at a speed of 1 mm/min and the maximum force (N) was calculated. Each test was repeated 10 times for each group, and a mean value was calculated.[18,23]

Statistical analysis

Statistical analysis was performed using SPSS version 22.0 software. Once the distribution Levene test) and homogeneity (Shapiro-Wilks test) of the data were verified, the statistical tests were performed. For microbiological analysis, the Kruskal-Wallis test and the Dunn post-test were used; for analysis of adhesive strength, data were submitted to two-way ANOVA and Bonferroni post-test (α=.05).

RESULTS

Microbiological analysis

The CFU/mL count of *C. albicans* varied according to the group (Table 2). There was a reduction in biofilm formation by associating nystatin with Ultra Corega Cream Adhesive *(p*<.001).

Table 2. Comparison of colony-forming unit count (CFU/mL) in $\log_{10}$ under different experimental conditions

	***C. albicans* (CFU/mL)**
PMMA (No Adhesive)	8.38 [8.18;8.58] ^{A}
PMMA + Ultra Corega Cream Adhesive	8.32 [8.08;8.57]^{A}
PMMA + Ultra Corega Cream Adhesive + Nystatin	5.01 [3.47;6.55]^{B}

Data are expressed in median [Confidence Interval] (n=10). *Different letters indicate differences between groups. Kruskal-Wallis followed by Dunn's post-test. *p*<.05.
Source: Own authorship.

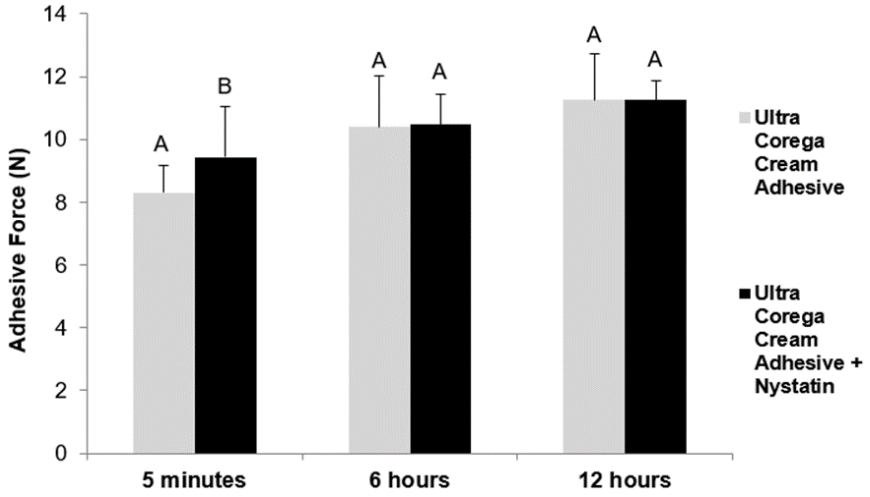
Adhesive force

When considering the type of material, independently, the adhesive strength of Ultra Corega Cream Adhesive (9.98±1.82N) maintained with the incorporation of nystatin (10.38±1.34N) (p=.226).

The time factor, independently, influenced the adhesive strength of the materials, which was lower after 5 minutes of application (8.86±1.40N), compared with 6 hours (10.42±1.30N) (p=.001) and 12 hours (11.25±1.09N) (p<.001).

The adhesive strength of Ultra Corega Cream Adhesive modified with nystatin was higher than that of the commercial product after 5 minutes of application (p=.048). There was no significant difference in the other times evaluated (p>.05) (Fig. 2).

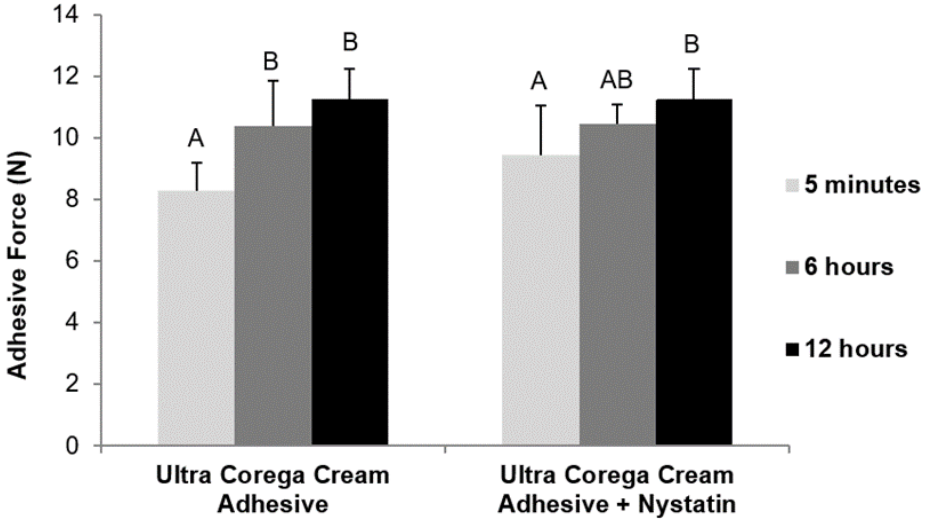
Figure 2. Comparison of the adhesive force (N) of the different materials at the same time



Source: Own authorship.

Both groups presented changes in adhesive strength as a function of time (p<.05). For the commercial Ultra Corega Cream Adhesive there was a significant difference after 5 minutes of application, when compared to 6 hours and 12 hours, which in turn were similar to each other (p=.392). With the incorporation of nystatin, the lowest adhesive strength was observed with 5 minutes of application, after 6 hours the values were intermediate, and after 12 hours, higher (Fig. 3).

Figure 3. Comparison of the adhesive force (N) of each material at different times



Source: Own authorship.

DISCUSSION

The results of this study rejected the null hypothesis, because significant differences were found in the biofilm formation and in the adhesive strength of the experimental groups.

Studies show that many users of removable prostheses use some type of adhesive to improve retention, stability and quality of life.[24-25] These materials are compositions with mucoadhesive characteristics, available in different formulations, and may be soluble (creams and powders) or insoluble (tapes).[19]

Candidiasis is an opportunistic fungal infection. Patients with low immunity, those who have undergone radiation, people with cancer, HIV infection or prolonged treatment with antibiotics are sensitive to infections.[26] Among the risk factors for its development, we can mention the reduction of salivary flow, the use of dental prostheses, medications, smoking, stress, diabetes, among others. These infections can be harmful if treatment with antifungals is not performed.[27]

The literature suggests that most drugs available for the treatment of oral lesions caused by *C. albicans* are effective. However, the responses of microorganisms to environmental stresses have evolved, that is, new survival strategies are involved, which increases the frequency of acquisition of drug resistance.[28] High rates of resistance are observed for antifungals tested in infections caused by *C. albicans*, which are mostly associated with the Azores. Resistance to these drugs can occur through different mechanisms such as activation of efflux pumps; ERG-11 gene mutation; dysregulation of ERG-11 gene expression; and changes that affect the ergosterol biosynthesis pathway.[29] Therefore, the treatment of choice, including for patients with systemic comorbidities, is the topical use of nystatin, due to the lowest resistance rates.[30]

The use of nystatin is recommended for the treatment of some fungal infections, such as oral candidiasis, in which it must be administered several times a day as it has reduced bioavailability,[31] and is generally prescribed at a concentration of 100.000 IU/mL at doses of 5 mL, four times a day.[27,32-33] However, it is not enough just to treat the tissue, the prosthesis should also be conditioned, for this reason, the addition of antimicrobial agents such as nystatin to adhesives can help in the treatment of these diseases.[34]

Studies have proposed incorporating antifungal agents into dental materials, such as tissue conditioners in an attempt to maintain the effective concentration of the drug at the infected site, reducing dissolution and early elimination from the oral cavity.[12,35-36] It has been demonstrated that when incorporated into a tissue conditioner, the antifungal agents chlorhexidine, clotrimazole, fluconazole and nystatin inhibit the growth of *C. albicans*. However, this association modified the physical and mechanical properties of the material.[28] On the other hand, another study reported that the bond strength between the denture base and a soft coating modified with antifungals, including nystatin, was not affected.[34]

The incorporation of nystatin in resilient materials inhibits fungal growth for 14 days.[12] A study suggests that the incorporation of miconazole nitrate in adhesives for dental prostheses can be an effective strategy without changing the adhesive strength, with great potential for the treatment of denture stomatitis.[22]

Despite the growing popularity of dental prosthesis adhesives, the scientific basis related to the effect of the incorporation of this antifungal in its properties is scarce. According to the results of the present study, the incorporation of nystatin in the adhesive promoted a reduction of 3 logs in the count of *C. albicans*, and can be used as an adjuvant in the treatment of oral or pharyngeal candidiasis, via release of the antifungal agent.

When using prosthetic adhesives, they are expected to provide retention and stability to the denture over a period of time. The method used in

this study to evaluate adhesive strength is advantageous because it is simple and requires no special equipment.[18,22]

By applying a thin layer of the adhesive to the inner surface of the denture, and then inserting it into the oral cavity, the hydrophilic compounds absorb and hold water to improve absorption strength and the hydrophobic compounds prevent excessive swelling and dissolution.[18,37-39]

In this study, the adhesive strength of Ultra Corega Cream incorporated with nystatin was higher after 5 minutes of application compared to the commercial product, and in the other times there was no significant difference, and could give safety and comfort to the patient for at least 12 hours.

The results of this study demonstrate that the incorporation of nystatin can be a viable alternative favoring the delivery of drugs to the patient, without negative alteration in the adhesive strength of the adhesive for dental prosthesis. However, further studies are needed to obtain more information about this proposal, such as evaluations in composite biofilms, mucoadhesive test and antifungal release mechanism.

CONCLUSION

Based on these findings, the use of prosthetic adhesive may be a good way of releasing the antifungal agent into the oral cavity, since the incorporation of nystatin reduced biofilm accumulation with a positive influence on the initial adhesive strength.

RESUMO

Introdução: A base científica relacionada com o efeito da incorporação de antifúngicos nas propriedades dos adesivos para próteses dentárias é escassa. **Objetivo:** Este estudo incorporou um agente antifúngico em um adesivo e avaliou a influência na formação do biofilme e na resistência adesiva. **Delineamento:** Espécimes em resina à base de polimetilmetacrilato (PMMA) foram divididos em grupos: PMMA (Sem Adesivo); PMMA+Ultra Corega Creme Adesivo e PMMA+Ultra Corega Creme Adesivo+Nistatina. O biofilme de *Candida albicans* foi cultivado nos espécimes e a viabilidade celular foi investigada através da contagem de unidades formadoras de colônias (UFC/mL). A força adesiva foi testada após 5 minutos, 6 e 12 horas. Para a análise microbiológica, os dados foram avaliados por Kruskal-Wallis e pós-teste de Dunn, e para a análise da força adesiva por ANOVA de duas vias e pós-teste de Bonferroni (α=.05). **Resultados:** Houve uma redução na formação de biofilme na superfície do adesivo modificado com nistatina (p<.001). Após 5 minutos, a força adesiva do Ultra Corega Creme modificado foi maior que a do produto comercial (p=.048), não havendo diferença significativa nos demais tempos (p>.05). **Implicações:** A incorporação de nistatina no adesivo protético reduziu o acúmulo de biofilme, com uma influência positiva na força adesiva inicial, podendo ser uma alternativa viável para a entrega do fármaco ao paciente.

DESCRITORES

Adesivos; *Candida albicans*; Prótese Dentária; Nistatina.

RESUMEN

Introducción: La base científica relativa al efecto de la incorporación de agentes antifúngicos en las propiedades de los adhesivos para prótesis dentales es escasa. **Objetivo:** En este estudio se incorporó un agente antifúngico a un adhesivo y se evaluó su influencia en la formación de biofilm y la resistencia adhesiva. **Delineación:** Las muestras de resina de polimetilmetacrilato (PMMA) se dividieron en grupos: PMMA (Sin adhesivo); PMMA+Adhesivo en crema Ultra Corega y PMMA+Adhesivo en crema Ultra Corega+Nistatina. Se cultivó biofilm de Candida albicans en las muestras y se investigó la viabilidad celular mediante el recuento de unidades formadoras de colonias (UFC/mL). La fuerza adhesiva se comprobó después de 5 minutos, 6 y 12 horas. Para el análisis microbiológico, los datos se evaluaron mediante Kruskal-Wallis y postest de Dunn, y para el análisis de la resistencia adhesiva mediante ANOVA de dos vías y postest de Bonferroni (α=,05). **Resultados:** Se redujo la formación de biopelículas en la superficie del adhesivo modificado con nistatina (p<0,001). Después de 5 minutos, la fuerza adhesiva del Ultra Corega Creme modificado fue mayor que la del producto comercial (p=.048), sin diferencias significativas en los otros tiempos (p>.05). **Implicaciones:** La incorporación de nistatina en el adhesivo protésico redujo la acumulación de biofilm, con una influencia positiva en la resistencia adhesiva inicial, y podría ser una alternativa viable para administrar el fármaco al paciente.

DESCRIPTORES

Adhesivos; *Candida albicans*; Prótesis Dental; Nistatina.

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COLLABORATIONS

GCA: Data collection, analysis of results and writing of the manuscript. CBS and VCO: Data collection and review of the manuscript. ACR end EW: Analysis of results and manuscript review. CPL and DTC: Study design, analysis of results and manuscript review. **All authors agree and are responsible for the content of this version of the manuscript to be published.**

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AVAILABILITY OF DATA

Under the confidential responsibility of the authors responsible.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.